

## Research Article

# Feto-Maternal Factors and Yield of Stem Cells from Umbilical Cord Blood (UCB): Experiences from South India

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### Abstract

**Introduction:** Umbilical Cord Blood (UCB) is an alternate source of stem cells with lesser immunogenicity. As harvesting of UCB stem cells is exhaustive and expensive, feto-maternal factors that result in better yield are to be characterized.

**Objectives:** To analyze the feto-maternal factors in relation to the yield of stem cells.

**Materials and Methods:** A total of 57 UCB units were collected by ex-utero procedure and yield of stem cells was assessed. The feto-maternal factors, namely maternal age, birth weight, duration of gestation, placental weight and length of cord were correlated with yield.

**Results:** The mean volume of UCB was 45.3 ml (range 35-50 ml), excluding anticoagulant. Birth weight and placental weight had positive correlation with yield, with a weightage of 20% each ( $P < 0.05$ ). Maternal age showed negative correlation with yield of Total Nucleated Cells (TNCs). Gestational age and cord length showed no significant correlation. The yield was as follows; Mean TNCs ( $\times 10^8$ ) 4.17  $\pm$  1.01, mean CD34+ cells ( $\times 10^6$ ) 2.14  $\pm$  1.8 and mean CFU-GM cells/ml ( $\times 10^4$ ) 2.01  $\pm$  0.61. The final yield of CFUs was 20%.

**Conclusion:** Feto-maternal factors; higher birth weight and placental weight had a good positive correlation with the yield of UCB stem cells. The efficiency rate was 20%, which is promising, since the UCB volume, mean birth weight and placental weight were lower than other similar studies. In large scale UCB banking, these positive factors are recommended as criteria for selection of stored UCB units for processing and transplantation.

**Keywords:** CD34+ Progenitor Cells; CFU-GM; Feto-Maternal Factors; Total Nucleated Cells (TNCs); Umbilical Cord Blood (UCB); UCB Stem Cells

### Introduction

Full term babies are estimated to have a wealth of Total Nucleated Cells (TNCs), CD34+ progenitor cells and Colony Forming Units (CFU-GM) in their Umbilical Cord Blood (UCB). From placenta, which is generally considered a bio-waste, these stem cells can be harvested with no risk for the mother or the baby. These cells are least immunogenic, can bypass HLA mismatch issues and so has the least risk of Graft vs. Host Disease (GVHD).

There are many UCB banks, both in private and public domain. But, processing, freezing and retrieving stem cells are tedious and expensive. There are no uniform policy or uniform standards for UCB cell processing. Hence, a study was undertaken to analyze feto-maternal factors in relation to the yield of stem cells.

### Materials and Methods

UCB was collected from singleton, full term, low risk vaginal deliveries as per standard obstetric practices from SAT Hospital, Govt. Medical College, Thiruvananthapuram, Kerala, South India. Ex-utero collection of UCB was done with strict aseptic precautions by trained obstetric staff. The expelled placenta was

placed on a sterile sheet on an elevated stand. The cord above the clamp was sterilized with povidone iodine and a 16-G needle from a blood collection bag with 20 ml of CPD as anticoagulant was inserted into the umbilical vein and UCB could flow by gravity till the flow ceased. The UCB bags were stored at 4-8°C till it was transferred to the processing unit within 24 hours at Rajiv Gandhi Center for Biotechnology (RGCB), Thiruvananthapuram. Socio-demographic and obstetric details were collected using a Performa. Institutional Research and Ethics Committee approval from both the institutions and informed consent from the mothers were obtained prior to the study.

The volume of UCB was noted and the UCB units were frozen in a -80°C freezer and then transferred to -195°C in liquid nitrogen. Three methods for isolation of TNCs were piloted before the study; pure cell select system filtration method and Percoll as well as Ficoll gradient centrifugation along with magnetic cell sorting. The Percoll method with magnetic cell sorting, which gave the best yield was used in the study. RBCs were lysed using Erythrocyte lysing solution. The isolated TNCs were plated in non-coated tissue culture flasks in IMDM expansion medium containing 20% FBS, 10 ng/ml bFGF, 2 mcg/ml heparin, 100 U penicillin and 1000 U streptomycin. CD34+ cells were then allowed to adhere overnight and non-adherent cells were washed out. For CFU-GM assay, cells were cultured as per standard procedures [1]. Colonies, that defined as clusters containing at least 40 cells after 14 days culture were scored. The cells after passage No 2 were characterized by immunophenotyping.

The data was computed and analyzed using SPSS Version 16. Descriptive statistics was used for participant characteristics and univariate analysis was done for significance. A significance level of  $P < 0.05$  was accepted.

## Results

A total of 57 UCB units were collected, but only 45 could be processed. The baseline feto-maternal factors are detailed in Table 1. The mean volume of UCB was 45.3 ml (range 35-50 ml), excluding anticoagulant. The mean maternal age was 29.51 years, with a range of 19-37 years. The mean birth weight was 2.81 Kg. The yield of TNCs, CD34+ cells and Colony Forming Units (CFU-GM) are depicted in Table 2. The yield was as follows; mean TNCs ( $\times 10^8$ ), number 4.17  $\pm$  1.01 (range 1.53-5.97), mean CD34+ cells ( $\times 10^6$ ) number 2.14  $\pm$  1.8 (range 1.20- 8.32) and mean CFU-GM cells/ml ( $\times 10^4$ ) number 2.01  $\pm$  0.61 (range 1.21- 3.02). Correlation with five variables namely, maternal age, birth weight, duration of gestation, placental weight and length of cord were analyzed and are detailed in Table 3. Birth weight and placental weight had a positive correlation with yield, with a weightage of 20% each ( $P < 0.01$ ). Maternal age showed negative correlation with yield of total nucleated cells (TNCs). Gestational age and cord length showed no significant correlation ( $P > 0.05$ ).

Parameter	Mean (SD)	Median	Range
Maternal Age (yrs.)	27.00 (5.09)	25	19-37
Duration of Gestation (wks.)	37.93 (1.30)	38	5-41
Birth Weight of Baby (Kg)	2.81 (2.78)	2.78	1.97-3.9
Weight of placenta (g)	466.48 (73.91)	461	325-644
Length of cord (cm)	54.71 (1.68)	45	35-50

**Table 1:** Feto-Maternal Factors in the Study.

Parameter	Mean (SD)	Median	Range
UCB net Volume (ml)	45.33	45	35-50
TNCs ( $\times 10^8$ ), number	4.17 (1.01)	4.23	1.53-5.97
CD34+ cells ( $\times 10^6$ ) number	2.14 (1.8)	2.98	1.20- 8.32
CFU-GM cells/ml ( $\times 10^4$ ) number	2.01 (0.61)	2.08	1.21- 3.02

**Table 2:** Yield of Stem Cells from UCB Units.

Parameter	TNCs ( $\times 10^8$ ), number Spearman Correlation (r) (P Value)	CD34+ cells ( $\times 10^6$ ) number Spearman Correlation (r) (P Value)	CFU-GM cells/ml ( $\times 10^4$ ) number Spearman Correlation (r) (P Value)
Maternal Age (yrs.)	-0.309 (0.039) *	-0.252 -0.06	-0.3 -0.07
Duration of Gestation (wks.)	-0.202 -0.184	-0.08 -0.09	-0.04 -0.33
Birth Weight of Baby (Kg)	0.447 (0.002) *	0.15 (0.01) *	0.17 (0.03) *
Weight of placenta (g)	0.448 (0.002) *	0.09 (0.02) *	0.09 (0.4) *
Length of cord (cm)	0.251 -0.097	0.12 -0.07	0.08 -0.06

**Table 3:** Correlation of Feto-Maternal factors and Yield by Univariate Analysis.

The Percoll method with magnetic cell sorting was found give better yield than Ficoll method and pure cell filter separation method. The yield of CFUs was 20%; colonies appeared by 7th day, slowly expanded and became confluent by 1 month. The cells after passage No 2 were characterized by immunophenotyping. The confluent flasks were then treated for cryopreservation or used for passage and characterization in two ongoing projects in RGCB, Thiruvananthapuram as the following; neuronal cell differentiation as Retinal Ganglion Cells (RGCs) for cell replacement in glaucoma and Vascular Endothelial Cells (VECs) for vascular graft tissue engineering.

## Discussion

Feto-maternal factors that influence the yield of TNCs, CD34+ cells and CFU-GM were evaluated in this study. The socio-economic status of the mothers was comparable in the study and the women had no addictions like alcoholism, smoking or substance abuse. The donor parameters are known to influence yield [2]. Collection, characterization and storage of UCB stem cells was found to be expensive and exhaustive. The initial bacterial contamination rate could be reduced to <5% by careful training and standardization of the procedure. The mean volume of UCB in the present study was 45.3 ml, excluding the anticoagulant and the range was 35-50 ml. In a previous Indian study, the volume reported was 83.3 ml (range 30-140), including 22 ml of anticoagulant [3]. A higher mean volume of 60 ml, excluding the anticoagulant (range 20-182 ml), has been reported by the Japanese cord blood bank [4]. However, they proceed processing only if volume is more than 32 ml and the US use a volume of 40 ml, excluding anticoagulant solution. As the yield of cells is based on the volume [5], the yield from lesser volume noted in the present is of practical significance, compared to studies from other countries. Out of the five variables studied, namely maternal age, birth weight, duration of gestation, placental weight and length of cord, birth weight and placental weight had a positive correlation with yield, with a weightage of 20% each. This observation is in accordance with other workers, who have reported that bigger is better [6-8]. A birth weight of >3200 g and placental weight of >700 g is generally accepted to give better yield, along with other factors [9,10]. An Indian study had reported UCB volume and weight of baby and placenta as the determinants [3]. In the present study, the yield of TNCs had a negative correlation with maternal age, as reported by the Japanese cord blood bank study [6]. However, maternal age >25 years, prolonged labor, longer cord, prematurity and fewer parity have also been reported to give better yield by some workers [9,10]. Presence of meconium in amniotic fluid is another factor that has been reported [5]. This situation occurs only in fetal distress and was not applicable in the present study. Other factors like gender of baby; female sex was associated with more TNC and male sex with more CD34+ cells and higher birth order have been reported by some workers to increase yield [11-13], but our study did not show similar result.

The comparison between the present study and the Japanese cord blood network was as follows; mean TNCs (x 108), number 4.17 +/- 1.01 (range 1.53-5.97) vs. 6.45 +/- 2.70 (range 2-27.3), mean CD34+ cells (x106) number 2.14 +/- 1.8 (range 1.2- 8.32) vs. 2.28 +/- 1.95 (range 0.17-15.3) and mean CFU-GM cells/ml (x 104) number 2.01 +/- 0.61 (range 1.21- 3.02) vs. 2.25 +/- 2.48 (range 0.00-18.2) respectively [4]. Assessing TNCs is easy, but reaping CD 34+ cells and growing them takes long and sensitive procedures [1,14,15]. TNCs may contain live/ dead cells and nu-

cleated RBCs as well. CD 34 is a protein antigen present on stem cells. CD34 expression is an indirect indicator of stem cell numbers and CD 34 assay is easy to perform. But, only about 10-20% of CD 34+ cells can multiply to produce new cells. Thus, measuring CD34 + cells alone over-estimates the quantity of stem cells in UCB units. CD 34+ assay does not guarantee that the cells are healthy. These may be damaged during collection and processing. The quality of the cells can be established by CFU assay, which can be done only after successful culture and may take several weeks. Even though, CD 34+ assay is generally considered as a surrogate marker of engraftment and the result available within a few hours of processing, the CFU is the best measure of viability and successful engraftment, but the result available only after a few weeks [16,17].

In the present study, Percoll gradient centrifugation method was found to yield maximum TNCs, compared to Ficoll and Pure cell filter separation methods. It has been reported that different collection and processing methods result in variable yield [18]. Out of the 45 units, 9 could be expanded, characterized and cryopreserved as CFUs. A recovery rate of 51% has been reported by the Japanese cord blood network [6], compared to a yield of 20% in the present study. The lesser volume of UCB collected and the lack of previous experiences in processing may be the reason for the lesser yield in the present study. However, the efficiency rate of 20% is promising, since the UCB volume, mean birth weight and placental weight were lower than other similar studies.

In the present study, the cells after passage No 2 were used for characterization by immunophenotyping. It has been reported that UCB CD 34+ cells are capable of at least 5 serial replatings in vitro [15-17]. It is interesting that the cells in the study were used in two ongoing projects in RGCB, Thiruvananthapuram. One was in the neuronal cell differentiation as Retinal Ganglion Cells (RGCs) for stem cell replacement in glaucoma [19] and the second was for generation of Vascular Endothelial Cells (VECs) for vascular graft tissue engineering [20,21].

## Conclusion

Feto-maternal factors, especially higher birth weight and placental weight had a positive correlation with the yield of UCB stem cells. The efficiency rate was 20%, which is promising, since the UCB volume, mean birth weight and placental weight were lower than other similar studies. In large scale UCB banking, these positive factors can be used as criteria to select the stored UCB units for processing and transplant utilization.

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